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(54) Title: EXTRACTION OF BETA-GLUCAN FROM CEREALS

(57) Abstract: A process for the extraction of f3-glucan from cereal including the steps of: (a) heating cereal including about 5% to about 30% by weight water at a temperature between about 50 and about 150 °C, and maintaining the water content of the cereal above about 5% by weight, for a time sufficient to deactivate enzymes in the cereal capable of starch hydrolysis; 10 (b) adding water to the cereal to form a slurry of an aqueous solution of f3-glucan and a solid residue; and (c) separating the aqueous solution from the solid residue.

EXTRACTION OF β-GLUCAN FROM CEREALS

FIELD OF INVENTION

This invention relates to a process for extracting β-glucan from cereals. In particular, the invention relates to a process for extracting β-glucan from barley flour where the flour is heated at high humidity to deactivate enzymes present in the flour which enzymes would otherwise degrade starch to soluble maltose or malto-oligosaccharides. The invention also relates to heat-treated flour, and the aqueous β-glucan extract formed during the process and to β-glucan when extracted by the process.

BACKGROUND

The term "β-glucan" refers to those polysaccharides which comprise D-glucopyranosyl units which are linked together by (1→3) or (1→4) β-linkages. β-Glucans occur naturally in many cereal grains such as oats and barley. The molecular weight of β-glucan molecules occurring in cereals is typically 200 to 2000 kiloDaltons.

β-glucan is desirable as a food additive, for example, to impart texture ("mouth feel") to foods or useful as edible films for food coatings. β-glucan may also be used to add bulk to foods and has the advantage of having a neutral flavour.

β-glucan is also desirable as a therapeutic agent. There is evidence that β-glucan can lower serum cholesterol levels, heal wounds, moderate glycaemic response, and alleviate constipation. β-Glucan can actively bind to specific cell receptors and therefore may be useful for the treatment of a wide variety of disorders or diseases.

The known methods for extracting β-glucan from cereal grains, such as oats and barley, involve several steps. Firstly, the cereal grain is milled to a flour prior to extracting β-glucan from the flour using warm or hot water or an aqueous alkali solution. The milling step facilitates release of the β-glucan from the cereal. The aqueous extract of β-glucan is then separated from the solid flour residue. Finally, the β-glucan is recovered from the extract.

The known methods of recovering the β-glucan from the aqueous extract include precipitation of the β-glucan using a water miscible solvent, such as alcohol, or by freezing and then thawing the extract to give a precipitate of β-glucan which can be recovered by filtration or centrifugation, or by drying the extract to remove the water. The extraction of the β-glucan itself from the cereal is not generally a costly process. However, the recovery of the β-glucan from the extract is costly. This is due to the large amounts of water that must be removed to give solid β-glucan.

The extract recovered from aqueous extraction of the cereal typically contains water soluble impurities as well as the desired β-glucan. Starch present in the cereal has low solubility in water and therefore does not usually constitute a problem impurity in the aqueous extract. However, enzymes are present in the cereal which hydrolyse the starch to water soluble maltose and malto-oligosaccharides. The maltose and malto-oligosaccharides are a significant impurity in known processes for obtaining β-glucan by aqueous extraction of cereal.

In addition, it is difficult to control the molecular weight of the β-glucan product obtained from known processes. High molecular weight β-glucan is preferable for certain uses. For example, high molecular weight β-glucan is preferable for moderating glycaemic response and for lowering serum

cholesterol levels. On the other hand, low molecular weight β-glucan may be preferable as a food additive. For example, low molecular weight β-glucan can form a gel having beneficial textural properties for processed foods.

In order to obtain a high molecular weight β-glucan product, previous methods of β-glucan extraction from cereals have required that enzymes present in the cereal be deactivated prior to the extraction step. The enzymes are responsible for lowering the average molecular weight of the β-glucan. They are deactivated either by treating the flour with boiling ethanol/water mixtures or by treating the flour with an aqueous acid solution.

It is also known to simply heat flour. Heating flour on its own has been employed for certain purposes, such as to improve the strength of dough formed from the flour.

US patent 4804545, for example, discloses a method of inactivation of natural enzymes in barley flour by heating a waxy barley of reduced particle size preferably for 1 to 2 hours. However, this treatment only deactivates a small amount of the amylases in barley flours resulting in the continued presence of maltose and malto-oligosaccharides resulting from starch hydrolysis. US patent 6083547 also refers to heating flour in a moist environment. However this process disclosed is directed, *inter alia*, to deactivation of enzymes that hydrolyse β-glucan only, thus starch hydrolysis is not much affected.

The inventor has found that heat treatment of the flour under humid, moist, conditions is effective in deactivating enzymes present in the flour that are capable of hydrolysing starch and β-glucan. The process disclosed in US 4804545 does not disclose the need for moisture in the heating process, nor does it disclose the levels of water needed to achieve effective enzyme deactivation. The process of US 6083547 does not teach deactivation of

amylases. The β-glucan extracted from the treated flour by the process of the present invention is less contaminated by maltose and malto-oligosaccharides and it is possible to obtain β-glucan having a high average molecular weight.

It is therefore an object of this invention to provide an improved process for extracting β-glucan, or to at least provide a useful alternative.

SUMMARY OF INVENTION

In a first aspect the invention provides a process fro the extraction of β-glucan from cereal including the steps of:

(a) heating cereal including about 5% to about 30% by weight water at a temperature between about 50 and about 150°C, and maintaining the water content of the cereal above about 5% by weight, for a time sufficient to deactivate the enzymes in the cereal capable of starch hydrolysis;

(b) adding water to the cereal to form a slurry of an aqueous solution of β-glucan and a solid residue; and

(c) separating the aqueous solution from the solid residue.

Preferably water is added to the cereal in an amount sufficient to achieve 15% to 30% by weight water in the cereal.

Preferably the cereal is allowed to cool to a temperature below about 50°C before adding water to form the slurry.

In another aspect of the invention there is provided a process for the extraction of β-glucan from cereal including the steps:

(a) heating cereal including about 5% to about 30% by weight water at a temperature about of 50 to about 150°C, and

maintaining the water content of the cereal above about 5% by weight, for a time sufficient to deactivate the enzymes in the mixture capable of starch hydrolysis;

(b) allowing the cereal to cool to a temperature below approximately 50°C;

(c) mixing the cereal with water to form a slurry of an aqueous solution of β-glucan and a solid residue; and

(d) separating the aqueous solution from the solid residue.

It is preferred that the amount of water present during the heating step in (a) is approximately 15 to 25% by weight of the cereal. It is also preferred that the temperature of the heating step in (a) is in the range of approximately 60 to 100°C, preferably between 80 and 95°C.

Preferably the cereal is heated in step (a) for between about 1 hour and 10 hours, more preferably between about 2 and about 10 hours, most preferably between about 3 and about 10 hours.

Preferably the cereal is heated in step (a) for between 1 and 5 hours at 90°C.

It is also preferred that the cereal is heated in a sealed container to prevent loss of water.

Preferably the cereal is dried after heating step (a).

Preferably the cereal used is in the form of a flour or a flour fraction, for example where starch has been sieved out.

It is also preferred that the cereal, particularly when in the form of flour or a flour fraction, is washed with cold water after the heating step (a) to remove water soluble impurities, such as sucrose and soluble protein.

Preferably the slurry is formed with water at a temperature of about 60°C or less.

Preferably the slurry is held for between about 30 minutes and about 2 hours before the separation step.

The preferred cereal is barley.

In a preferred embodiment of the invention the process also involves the addition of enzymes to the slurry, during aqueous β-glucan extraction, which enzymes are capable of hydrolysing the β-glucan to β-glucan of lower average molecular weight. The amount of enzymes added can be used to control the average molecular weight of the β-glucan recovered. Preferably the enzymes are cellulases.

In another preferred embodiment of the invention the process also includes recovering the β-glucan from the aqueous solution separated from the solid residue. This may be by evaporation, for example spray drying, or by cooling to form a gel, or by any other known method.

In another aspect of the invention there is provided β-glucan obtained by the process of this invention.

In another aspect of the invention, there is provided a method of regulating glycaemic response or cholesterol levels in an animal comprising administering to the animal β-glucan obtained using the process of this invention.

Preferably the β-glucan is administered as part of a food product, such as breads or the like.

In another aspect the invention provides a food additive, including low molecular weight β-glucan when formed using the process of the invention.

In another aspect of this invention, there is provided a flour which has been heated in the presence of water according to the heat treatment step of the process of the first aspect of this invention.

In another aspect the invention provides a β-glucan aqueous solution, when produced by the process of the invention.

DETAILED DESCRIPTION

β-glucan occurs naturally in a wide variety of cereals. The process of this invention is not limited to any particular cereal. However, preferred cereals are barley and oats. β-glucan extraction from cereals is known and generally this occurs using aqueous extraction methods.

During the aqueous extraction of β-glucan from cereal, however, other water soluble materials are also extracted. This can lead to β-glucan of low or variable purity. One of the major sources of impurity in recovered β-glucan is the starch that is present in cereal. Starch itself has low solubility in water (especially cold water) and is therefore not itself readily extracted. However, enzymes which are also present in the cereal become mobilised in the presence of sufficient water and hydrolyse the starch into maltose and malto-oligosaccharides. The maltose and malto-oligosaccharides are readily soluble in water and are therefore extracted into water along with β-glucan. Recovery of the β-glucan from the aqueous solution then typically gives a product contaminated by significant amounts of maltose and malto-oligosaccharides.

Removing starch from cereal can therefore be advantageous for reducing the amount of maltose and malto-oligosaccharides produced from the starch during the extraction of the β-glucan.

Various methods are known for complete or partial removal of starch from cereal grain. These methods can be used as a preliminary step prior to use of known β-glucan extraction methods or prior to use of the present method. These known methods for starch removal include dry milling and wet milling as will be known to the skilled person.

Wet milling with water has a disadvantage since about 30–50% of the cell wall β-glucan is soluble in water at a temperature of 25°C. However, only 10–20% of the cell wall β-glucan is soluble in ice-cold water. Similarly, little of the cell wall β-glucan is soluble in ethanol or ethanol/water mixtures or aqueous solutions of certain salts. Therefore, for wet milling, it is preferable to use cold water or ethanol/water mixtures or aqueous solutions of certain salts. If used prior to the process of the present invention, the wet milled flour would need to be dried to remove excess water.

Dry milling may also be used for removing starch. A large proportion of the starch can be removed from dry flour by sieving or air classification. The cell wall material containing the β-glucan mostly occurs as particles which are larger than the starch granules after milling. Consequently, the starch granules will pass through the sieve while cell wall material will be retained. Air classification will separate out the dense starch granules from the cell wall material. However, it is to be understood that these methods of separation are not 100% efficient and that the starch fraction will contain some cell wall material and the cell wall material will contain some starch.

The present invention provides an alternative aqueous extraction process for β-glucan which has the effect of reducing the amount of starch hydrolysed

during water extraction of β-glucan. Heating the cereal in the presence of water according to the process of this invention deactivates some or all of the enzymes (eg α and β amylases) responsible for hydrolysis of starch to maltose and malto-oligosaccharides. In barley, the starch is mainly hydrolysed by β-amylase. Therefore, the amount of maltose and malto-oligosaccharides extracted into the water at the time of β-glucan extraction is minimised. This leads to recovered β-glucan of higher purity than would be achievable in the absence of deactivation of the enzymes. The amylase enzymes are relatively heat stable and thus a relatively long heating time in the presence of sufficient water is needed for deactivation of enzymes to affect starch hydrolysis in the process as described herein. Time periods of between about 1 and 10 hours are preferred to achieve sufficient deactivation. More preferably between 2, to 3, hours at about 90°C should be used.

The amount of water present is not critical but should be sufficient to create an environment in which the enzymes responsible for hydrolysis of starch are deactivated thus reducing production of starch hydrolysis products such as maltose and malto-oligosaccharides. At a temperature of 60-100°C, 5-15% by weight water in the cereal is considered effective with 15-25% preferred. Preferred temperature ranges are between 60°C and 95°C and more preferably between 80 and 95°C. It is preferred that the cereal is heated for between about 1 to 5 hours at a temperature of about 90°C. Excessive water is undesirable because this can lead to gelatinisation of starch at temperatures greater than about 60°C (hence the need to dry wet milled flour, as discussed earlier, if used, as an optional treatment step). In addition, too much water will result in premature extraction of the β-glucan and hydrolysis of starch to maltose and malto-oligosaccharides.

Cereals commonly include up to about 10-15% of water and this water needs to be maintained as far as possible in the cereal during the heating

step. To be effective the level of water in the cereal should be above about 5% by weight cereal throughout the heating step. It is preferred that water is added to the cereal to result in a cereal having between 15-25% water by weight cereal.

In order to maintain the amount of water in the mixture, it is desirable to carry out the heat treatment step in a sealed vessel or in a humid atmosphere to maintain the water content in the heated cereal at a level sufficient to deactivate the starch hydrolytic enzymes. As mentioned earlier a level of about 5% or more water by weight cereal is considered effective.

Optional steps are to dry the cereal and/or allow it to cool to below about 50°C, according to known methods following heating in the moist environment.

Following heating, the cereal can be washed in water to remove water soluble impurities, such as sucrose and water soluble protein. This step is optional but can increase the purity of the final β-glucan product produced. The water used is preferably cold water but warm water could also be used as would be known to the skilled person. The wash time is preferably between about 30 seconds to about 5 minutes. The step (if included) is completed in a manner which minimises loss of β-glucan. Hence a short wash time and cold water is preferred.

The β-glucan is then extracted from the heat-treated flour via aqueous extraction, preferably using hot water at a temperature up to about 60°C for a time between about 30 minutes to about 2 hours.

The β-glucan product can then be recovered from the aqueous extract by a variety of techniques, as will be known to the skilled person, such as evaporation techniques such as spray drying, or by cooling to form a gel.

Other known methods could also be used. It will be appreciated that the recovery step is additional and can be carried out at a time suitable to the user. Such an aqueous β-glucan product when formed by the process of the invention also forms an aspect of the present invention.

Separation of solid residue to leave the aqueous β-glucan extract is preferably achieved via centrifugation, however other known methods, such as filtration, can also be used.

The process of this invention can be varied to give different β-glucan products. The physical properties of a β-glucan product are dependent principally on the average molecular weight of the β-glucan molecules and the conformation of the β-glucan molecules. High molecular weight β-glucan is β-glucan having an average molecular weight greater than 5×10^5 Daltons. Low molecular weight β-glucan is β-glucan having an average molecular weight in the range of 5×10^3 to 2×10^5 Daltons. Since there are little or no β-glucan degrading enzymes left in the grain, it can be useful to add an enzyme, preferably a cellulase, to the extraction solution to partially degrade the β-glucan in a controlled fashion. β-Glucan having a desired average molecular weight range can therefore be obtained.

β-Glucan products can form a gel in water. The ease with which a β-glucan product forms a gel depends on the average molecular weight of the β-glucan and also depends on the manner in which a solution of β-glucan extracted from cereal grain is processed. Typically, high molecular weight β-glucan forms a viscous solution in water whereas low molecular weight β-glucan is more inclined to form a gel in water. Low molecular weight β-glucan can be used to provide textural properties in foods and drinks.

High molecular weight β-glucan is desirable for certain therapeutic uses because of its high viscosity in aqueous solution. The moderation of

glycaemic response and the lowering of serum cholesterol levels can be effected using β-glucan of high molecular weight. β-glucan can be included in food products such as breads, as will be known to a skilled person, for achieving these health benefits.

The heat treatment of the cereal also has the added benefit of denaturing protein in the cereal thus making the proteins less water soluble. As a result, these less soluble proteins are not extracted with the β-glucan.

It will be appreciated that the flour produced following heat treatment step used in the process according to this invention, can be dried to moisture contents below about 12% and then stored under conditions used for untreated flour. These treated flours may have specific uses such as in pastry flours, but can also be kept and processed for extraction of β-glucan at a later time. Such heat-treated flour also forms an aspect of the present invention.

The invention is now described with reference to the following examples, but is not to be construed as limited thereto.

EXAMPLES

In the following examples, β-glucan content was determined by a modification of the McCleary method (Megazyme* Mixed-Linkage Beta-Glucan Assay). Starch contents were measured by taking a sample of extract (100 µL) and adding NaAc buffer (1 ml, 200 mM, pH 4.5) and amyloglucosidase (25 µL, Megazyme* amyloglucosidase 200 U/ml) and heating at 50°C for 30 min. Glucose content of a sample of the hydrolysis mixture (50-200 µL) was determined using GOPOD and comparing the absorbance at 510 nm to a glucose standard containing 1 mg/ml of glucose, that had been similarly treated with GOPOD.

Molecular weights (Mw) of the β-glucans were determined by size exclusion chromatography and multi-angle-laser-light scattering on a Waters 2690 HPLC attached to a DAWN EOS.

Shearzyme is a xylanase and Celluclast is a cellulase obtained from Novo Nordisk, 2880 Bagsvaaerd, Denmark.

*Megazyme, Bray Business Park, Bray, Co. Wicklow, IRELAND.

Example 1 - Preparation of enriched barley fraction

Pearled barley (150 g, 20% pearlings) was milled to a fine flour and the flour passed through a 90 μm sieve. The overs on the 90 μm sieve (Fraction 1) was found to contain 10.57% β-glucan on a dry weight basis with a flour yield of 66.2 g.

A pearled barley was also prepared by repeated milling and sieving (Fraction 2). This fraction contained 15.24% β-glucan.

Example 2 - Heat treatment and extraction of Fraction 1

Fraction 1 (2 g) from Example 1 was placed in a sealed tube together with water (0.2 g). The water was not in direct contact with the flour. The tube was heated at 70°C for 15 h. The flour was observed to have absorbed the water. The heat-treated flour was then mixed with water (25 ml), Shearzyme (2 μL) and Celluclast (0.05 μL) and the β-glucan was extracted at 60°C for 45 min. The spent flour was removed by centrifugation at 3000 rpm for 5 min, and the extract lyophilised. For comparison, a sample of flour that had not been heat and moisture treated was extracted and lyophilised in a similar manner. β-Glucan and maltose/malto-oligosaccharide content of the lyophilised solid was measured. Results are shown in Table 1.

Table 1

Flour	*β-Glucan %*	*Maltose %*
Heat/moisture-treated	49.5	10
No treatment	32.1	48

The results indicate a 5-fold decrease in maltose/malto-oligosaccharide content and a 53% increase in β-glucan content in the lyophilised solid.

Example 3 - Variation in moisture contents, heating times and temperatures

Fraction 1 (0.2 g) from Example 1 was heat/moisture treated in a sealed tube at differing temperature and times with differing amounts of water according to Table 2. The treated flour was then extracted with water (2.5 ml), Shearzyme (0.2 µL) and Celluclast (0.005 µL) at 60°C for 45 min. The spent flour was removed by centrifugation at 3000 rpm for 3 min and the starch content of the extract determined. Results are shown in Table 2.

Table 2

Water added for heat treatment/µL	*Temperature of heat treatment*	*Time for heat treatment/h*	*Maltose Content/%*
20	70	2	2.27
40	70	2	2.16
20	70	8	1.19
40	70	8	0.89
20	80	2	1.09
20	80	4.5	0.83
0	80	4.5	2.75
0	No heat treatment	No heat treatment	3.42

The results indicate that all heat treatments are effective in decreasing amylase activity but the presence of additional moisture and longer heat-treatment times is more effective.

Example 4 - Heat treatment and extraction of Fraction 2

Fraction 2 (2 g) from Example 1 was placed in a sealed tube together with water (0.2 g). The water was not in direct contact with the flour. The tube was heated at 70 °C for 15 h. The flour was observed to have absorbed all the water. The heated-treated flour was then mixed with water (25 ml), Shearzyme (2 µL) and Celluclast (0.05 µL) and the β-glucan was extracted at 60°C for 45 min. The spent flour was removed by centrifugation at 3000 rpm for 5 min and the extract was lyophilised. β-glucan content of the lyophilised solids was found to be 53 % and maltose/malto-oligosaccharide content was found to be 11.3 %.

Example 5 - Viscosity and Molecular Weight of β-glucan extracted from heat/moisture treated flours

Fraction 1 (2 g) from Example 1 was placed in a sealed tube together with water (0.2 g). The water was not in direct contact with the flour. The tube was heated at 80°C for 5.5 h. The heat-treated flour was then mixed with water (25 ml) and the β-glucan was extracted at 60°C for 45 min. No additional enzymes were added. β-glucan was precipitated from the extract by addition of ethanol and the precipitate was oven-dried. An identical extraction was performed except that the flour was not heat-treated. Viscosity was determined on a 1% solution by weight of the β-glucan solid, formed by dissolving the β-glucan precipitates in water at 90°C. For the β-glucan isolated from the heat-treated flour the relative viscosity was found to be 8100 whereas for that isolated from the untreated flour the viscosity was found to be 751. The molecular weight was determined to be 400,000 for

β-glucan extracted from flour that was not heat-treated and 2,000,000 for β-glucan extracted from flour that was heat-treated.

Example 6 - Washing of heat-treated flours

Fraction 1 (2 g) from Example 1 was placed in a sealed tube together with water (20 g). The water was not in direct contact with the flour. The tube was heated at 95°C for 6 h. The heated-treated flour was then stirred with water (30 ml) at 15°C for 30 min and the mixture centrifuged. The solids pellet was dispersed in water (20 ml) at 59°C together with Shearzyme (2 μL) and Celluclast (0.05 μL) and the mixture was occasionally stirred for 45 min at 59°C. Solids were centrifuged (3000 rpm, 5 min) and the supernatant was lyophilised. The β-glucan content of the lyophilised solid was found to be 76%, and about 56% of the β-glucan in the flour was recovered in the lyophilised solid. The cold-water extract in contrast contained about 18% β-glucan.

Example 7 – Determination of rate for amylase deactivation

Samples of Fraction 1 (0.2 g) from Example 1 were each placed in a sealed tube together with water (0.02 g). Each sample was heated for times ranging from 0 to 23 h. The heated-treated samples were then stirred with water (2 ml) at 60°C for 45 min and the mixture centrifuged. The malto-oligosaccharide content of the supernatant was determined as a measure of the amount of amylase deactivation that had occurred. The rate for malto-oligosaccharide production (or amylase deactivation) was fitted to a first order rate equation of the form:

$$y - y_o = A \exp[-r*(t-t_o)]$$

where t is the time, r the rate constant, t_o the time for water absorption to occur (~2 h), and A and y_o are constants.

For flour heated-treated at 70°C, r = 0.35 h. But at 60°C, r = 0.097.

The amount of water added during heat-treatment was also varied and the rate constant determined. Results are reported in the Table 3 for flour samples treated at 70°C.

Table 3

% Water added in heat-treated	*Rate constant*
0	0.01
3.5	0.05
5	0.078
10	0.35
15	0.44
20	0.53

Results indicate that the heat-treatment of flour at 70°C with added moisture is about 35 times more effective in deactivating amylase than heat-treatment without added water.

Example 8

A barley flour enriched in β-glucan was prepared according to example 1. The flour (2 g)was mixed thoroughly with water (0.2 g) and then heated in a sealed tube at 85°C for 6 h. A sample of the heat-treated flour (0.22 g) was then mixed with 4 ml of ice-cold water for 1.5 min, after which the water was removed by centrifugation. The pellet remaining was weighed and extra water added to give a total volume of water of 2 ml. The pellet was dispersed in the water together with Celluclast 0.02 µL and the β-glucan extracted by heating at 57°C for 40 min. The spent flour was removed by centrifugation and the supernatant lyophilised to give a white solid. Analysis

of the solid showed that about 88% of the β-glucan had been extracted into the aqueous phase and that the β-glucan content of the solid was 62%.

Example 9

A barley flour enriched in β-glucan was prepared according to example 1. The flour (2 g)was mixed thoroughly with water (0.2 g) and then heated in a sealed tube at 95°C for 1.5 h. A sample of the heat-treated flour (0.22 g) was then mixed with 4 ml of ice-cold water for 30 s, centrifugation for another 30 s and then washed with another 4 ml of ice-cold water for 30 s. The mixture was then centrifuged for 1 min. The pellet remaining was weighed and extra water added to give a total volume of water of 2 ml. The pellet was dispersed in the water together with Celluclast 0.02 µL and the β-glucan extracted by heating at 55°C for 45 min. The spent flour was removed by centrifugation and the supernatant lyophilised to yield a white solid. Analysis of the solid showed that about 56% of the β-glucan had been recovered from the flour and that the β-glucan content of the solid was 66%.

Although the invention has been described by way of example, it should be appreciated that variations and modifications may be made without departing from the scope of the invention as defined in the attached claims. Furthermore, where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred in this specification.

WHAT WE CLAIM IS

1. A process for the extraction of β-glucan from cereal including the steps of:
   (a) heating cereal including about 5% to about 30% by weight water at a temperature between about 50 and about 150°C, and maintaining the water content of the cereal above about 5% by weight, for a time sufficient to deactivate enzymes in the cereal capable of starch hydrolysis;
   (b) adding water to the cereal to form a slurry of an aqueous solution of β-glucan and a solid residue; and
   (c) separating the aqueous solution from the solid residue.

2. A process according to claim 1 wherein water is added to the cereal in an amount sufficient to achieve 15% to 30% by weight water in the cereal prior to heating step (a).

3. The process according to claim 1 or 2 wherein the mixture is allowed to cool to a temperature below about 50°C before adding water to form the slurry.

4. A process for the extraction of β-glucan from cereal including the steps:
   (a) heating the cereal including about 5% to about 30% by weight water at a temperature of 50-150°C, and maintaining the water content of the cereal above about 5% by weight, for a time sufficient to deactivate the enzymes in the mixture capable of starch hydrolysis;
   (b) allowing the cereal to cool to a temperature below approximately 50°C;
   (c) mixing the cereal with water to form a slurry of an aqueous solution of β-glucan and a solid residue; and

(d) separating the aqueous solution from the solid residue.

5. The process according to any one of claims 1 to 4 wherein the amount of water present during the heating step (a) is approximately 15 to 25% by weight of the cereal.

6. The process according to any one of the preceding claims wherein the temperature of the heating step is in the range of approximately 60 to 100°C.

7. The process according to claim 6 wherein the temperature range is between 80 and 95°C.

8. The process according any one of the preceding claims wherein the cereal is heated in step (a) for between about 1 hour and 10 hours.

9. The process according to any one of preceding claims wherein the cereal in step (a) is heated for between 1 and 5 hours at 90°C.

10. The process according to any one of the preceding claims wherein the slurry is formed with water at a temperature of about 60°C or less.

11. The process according to any one of the preceding claims wherein the cereal/water mixture is heated in step (a) in a sealed container to prevent loss of water.

12. The process according to any one of the preceding claims wherein the cereal is dried after heating in step (a).

13. The process according to any one of the preceding claims wherein the cereal is washed with cold water after the heating step (a), or after

drying following step (a), to remove water soluble impurities.

14. The process according to any one of the preceding claims wherein the slurry is held for between about 30 minutes and about 2 hours before the separation step.

15. The process according to any one of the preceding claims wherein the cereal is in the form of a flour or a flour fraction.

16. The process according to any one of the preceding claims wherein the cereal is barley.

17. A process according to any one of the preceding claims further including the addition of an enzyme during the aqueous extraction of β-glucan, the enzymes being capable of hydrolysing the β-glucan to β-glucan of lower average molecular weight.

18. The process according to claim 17 wherein the added enzyme is a cellulase.

19. The process according to any one of the preceding claims further including recovering the β-glucan from the aqueous solution by evaporation or by cooling.

20. β-glucan when obtained by the process according to claim 19.

21. A method of regulating glycaemic response or cholesterol levels in an animal comprising administering to the animal β-glucan according to claim 20.

22. A method according to claim 21 wherein the β-glucan is administered as

part of a food product.

23. A food additive, including low molecular weight β-glucan when formed according to the process of claims 17 or 18.

24. A flour produced by heating a cereal in the presence of 5-30% by weight water at a temperature between 50 and 150°C, maintaining the water level above 5% by weight cereal, for a time sufficient to deactivate enzymes capable of starch hydrolysis, and then drying the flour.

25. The flour according to claim 24 wherein the flour has a moisture content below about 12%.

26. An aqueous β-glucan solution produced by the process according to any one of claims 1 to 18.

27. A process for the extraction of β-glucan from a cereal substantially as herein described with particular reference to any one of the examples.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ02/00129

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C08B 37/00; A23L 1/10; A61K 31/716

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN Files WPIDS, MEDLINE, CA: Keywords beta glucan, extract, deactiv?, inact?, enzym?, glucanase, heat treat, humid?, steam, hot water

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Korean Patent Abstract 20000261045 B1 (REPUBLIC OF KOREA RURAL DEVELOPMENT ADMINISTRATION) 15 April 2000. See abstract.	1-27
P,X	WO 01/57092 A (GRANATE SEED LTD et al.), 9 August 2001. See whole citation and particularly Example 17.	1-27
X	US 6113908 A (D. PATON et al.), 5 Septemeber 2000. See whole document and particularly column 2, lines 1 to 5.	1-27

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 September 2002	11 SEP 2002
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **L.F. MCCAFFERY** Telephone No : (02) 6283 2573

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ02/00129

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4804545 A (K. J. GOERING et al.), 14 February 1989. See whole document.	1-27
X	WO 86/01080 A (BARCO INC), 27 February 1986. See whole document.	1-27
X	WO 95/07628 A (L. LINDAHL et al.), 23 March 1995. See whole document.	1-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ02/00129

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	200157092	AU	36236/01				
US	6113908	AU	35470/00	EP	1162891		
US	4804545	AT	42176	AU	46786/85	CA	1242160
		DE	3569459	EP	192677	ES	545945
		JP	61502940	KR	8903736	NZ	213059
		WO	8601080				
WO	8601080	AT	42176	AU	46786/85	CA	1242160
		DE	3569459	EP	192677	ES	545945
		JP	61502940	KR	8903736	NZ	213059
		WO	8601080				
WO	9507628	AT	199486	AU	77135/94	CA	2170988
		DE	69426834	EP	731646	ES	2154684
		JP	9505204				

END OF ANNEX